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Chapter

The Potential Application of Nanoparticles on Grains during Storage: Part 1 – An Overview of Inhibition against Fungi and Mycotoxin Biosynthesis

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Abstract

Cereals and legumes are the major staples across the globe, thus providing nutrition to humans, and their by-products utilized as animal feeds. However, mycotoxins synthesized by fungi contaminate these grains on the field during cultivation and are transferred to the storage centers. These fungi infect and deteriorate stored grains, thereby tampering with food security. Moreover, the deterioration decreases nutrient content and alters the physicochemical properties of grains. The current conventional methods used to reduce grain contamination are becoming ineffective, coupled with the detrimental health effects it has on the consumer and to the environment. Herein, we present an overview of the use of nanoparticles (NPs) as an alternative and novel method of reducing mycotoxin biosynthesis due to their potent biocidal properties. Silver nanoparticles (AgNPs) are considered and have shown promising and effective fungicidal properties against important storage fungi, and pests hence could be utilized in the agriculture and food sector for a vast myriad of applications. These may help to either minimize/eradicate the exposure to the mycotoxins and its adverse health effects, hence contributing to the holistic growth and development of people.

Keywords: grains, mycotoxins, nanoparticles, biocidal activities, reactive oxygen species

1. Introduction

According to [1], microbial contamination of grains has resulted in a decrease in its nutritional quality, therefore, negatively affecting the productivity of humans (the workforce of a nation). Grains (cereals and legumes) are staple foods and widely consumed around the world due to their nutritional value and calories. Eating food prepared from contaminated grains could lead to malnutrition due to insufficient nutrients in the grains or food poisoning from mycotoxins.

The presence of these mycotoxins affects the safety, quality, and functional properties of grains. Moreover, the organoleptic properties of products made from these grains could also be altered because some fungi strains produce potent odors, which serve as an antibiotic against other microorganisms [2]. There have been several reports regarding microbial contamination of grains [3–5] and the mycotoxins produced by some of these organisms potentially pose a health risk to consumers.

The ability of fungi to penetrate grains, reside within the endosperm, and utilize nutrients makes conventional methods insufficient to deal with the menace [6]. Therefore, the fundamental problem remains unsolved. A convenient and practical approach where the nutritional quality, sensorial properties, color, and shelf life of the grains remain unchanged is warranted in curbing this menace. Therefore, we propose nanoparticles as the ultimate solution to the predicament mentioned above since they are known to exert potent biocidal activities against the vast myriad of microorganisms [7–17] involves in contaminating grains, hence could be utilized as antifungal agents during grain storage.

This chapter summarizes the microbial contamination of grains and the existing conventional methods employed to curb and or minimize this menace. Also, the potential application of silver nanoparticles as an alternative to the traditional techniques is discussed.

1.1 The economic importance of grains

Foodgrains could be cereals or legumes (pulses). The world leading cereal grains are wheat, barley, rice, maize, oats, rye, millet, and sorghum. Reports show that cereals are the dominant crops cultivated globally, with 2500 million tons harvested in 2011. The proportion of maize, rice, and wheat harvested is 883; 723; 704 million tons, respectively [18, 19]. Cereals are whole, hulled, cracked, rolled, or ground forms of products produced from various grains constituting staple foods for many localities globally. They contain a substantial amount of starch, a carbohydrate that provides dietary energy [20]. Also, cereals are utilized in feeding livestock. Huntington [21] reported a starch content of 72% for corn and sorghum, while 57, 58 and 77% for barley, oat and wheat. Thus could be utilized to feed ruminants due to their high energy values. The role of cereal grains in the world food supply cannot be undermined as it provides 75% of the calories and protein in the human diet [22]. In Russia, folks use cereals in brewing (beer, kvass), production of distillates, and food (i.e., sweets, cookies, porridge, among others).

The second most important family of crops are the legumes, used for their grains, and as forage [23]. Previous works [24–26] have reported that legume seeds contain protein, soluble and insoluble fiber, slowly digested starch, micro- and macronutrients, and vitamins, in addition to various bioactive phytochemicals such as flavonoids and other antioxidants which are beneficial to human health. Legumes complement proteins in cereals and contain 20–45% protein compared to 7–17% in cereals [27]. Grain legumes are also utilized in feeding livestock, either as a concentrated compound feed (in poultry production) or as whole-crop forage (in cattle, sheep, and pig production) [23]. The presence of antinutritional factors (ANFs) such as Kunitz trypsin inhibitor (KTI), Bowman-Birk inhibitor, and lectins in legumes limits their utilization by humans and in animal husbandry with exception to ruminants (i.e., cattle, sheep and goat), which can degrade ANFs due to the microbial fermentation in their stomach [28]. ANFs can decrease the nutritive value of legumes and cause health problems that may be fatal for both humans (if a substantial amount is consumed) and animals [29]. Nevertheless, various methods have been proposed to decrease the concentration of these ANFs [30–32]. Legumes are also utilized in feeding fish, thus limit the need for expensive fishmeal in the

pisciculture industry [33–35]. Therefore, the safety and quality of grain legumes ought to be screened before utilization to avoid any further complications due to ANFs and mycotoxins.

1.2 Sources of fungi contamination of grains

Microorganisms plays vital role in balancing the ecosystem; they aid in the digestion of food in humans; are utilized in the production of food (i.e., starter culture in brewing, cheese production, among others), and serve as a good source of vital enzymes (exogenous enzymes). Nevertheless, these microorganisms could cause problems such as food poisoning (due to some mycotoxins they secrete), food spoilage, and grain contamination.

The entire production process (sowing, harvesting, postharvest drying, and storage) of grains are possible sources of fungi contamination [36]. Dust, water, diseased plants, insects, soil, fertilizers, animal excreta, and environmental pollutants are possible origins of fungi cross contamination. The farmer, the processor, and the distributor could be a source of microbial contamination as well as contaminated farm machinery and unclean storage facilities (silos, etc.). According to [37], microbial contamination from the skin, mouth, and nose of food handlers could be directly introduce into the food chain. During drying, most farmers step on the grains with their Wellington boots, which is a possible route of introducing microorganism [38].

The microflora of grains mainly belong to the *Alternaria, Fusarium, Helminthosporium*, and *Cladosporium* families. Yeasts were isolated from grains; however, its load was less compared to mold [4]. Mechanical damage during harvesting or processing could serve as a route via which fungi could penetrate the endosperm of seeds, reproduce, and secrete mycotoxins (aflatoxins, etc.), rendering the food unsafe for human consumption. According to the International Commission on Microbiological Specifications for Foods [39], isolated fungi were mainly on the surface of the kernel; only a few species occupy the inner parts of the seeds due to damage. Birds could introduce fungi on grains by (1) feeding on crops in the field. This can introduce gut microbiota to these plants, which could subsequently be spread by rainwater. (2) Their feet could also aid to spread microbes by landing and picking up fungi spores from a diseased plant/crop to healthy ones. Bats, and insects (bees) could also aid the contamination of crops on the field, which can spread during harvesting.

According to [40], the primary cause of spoilage in stored grains in developed countries is attributed to fungi, because insects and rodents are controlled successfully. Factors such as high temperature, humidity, and poor storage conditions create a conducive environment for fungi to flourish and synthesize mycotoxins. These secondary metabolites can cause diseases in humans and animals. For instance, aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, fumonisins, HT-2, and T-2 are classes of mycotoxins produced by various fungus species [41, 42]. Grapes were found to be contaminated with ochratoxin A, thus contaminating any product processed from them (juice, wine, vinegar, and dried grapes) [3].

2. Factors promoting microbial growth and mycotoxin production

When deciding whether moisture, temperature, etc., affects the safety of grains, other factors should be considered to settle on a scientifically proven conclusion. Extrinsic factors (temperature, relative humidity, mechanical injury on seeds during harvest or processing, insects, and rodents infestation) are environmental and

physical factors surrounding the grains whereas those attributed to the characteristics of the grains are intrinsic factors (pH, acidity, nutrient composition, biological structure, moisture content/water activity, redox potential, naturally occurring and added antimicrobial factors). Details on how these factors contribute to or promote microbial contamination of grains are examined below.

2.1 Nutrient content

Every organism requires essential nutrients for growth and maintenance of metabolic functions. Hence, the type and concentration of nutrients needed depends on the class of microorganism. A source of energy, water, nitrogen, vitamins, minerals, and other compounds provide these nutrients. The growth of *Aspergillus flavus* on grains was significantly affected by the concentration of soluble sugars. Low sugar levels retarded its growth, whereas concentrations between 3.0 and 6.0% resulted in rapid growth, and the subsequent production of aflatoxin B1. Nevertheless, aflatoxin B1 production was significantly promoted due to the bioavailability of amino acids (arginine, glutamic acid, aspartic acid) and zinc in the grains [43]. In a similar study, Li et al. [44] reported different concentrations of mycotoxins (aflatoxin B1 (AFB), deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA)) on numerous swine feeds. These outcomes could be attributed to the nutritional composition of the feeds. The nutritional requirement of pigs depends on the state (gestating, finisher, grower, starter, etc.) hence varied feed rations are given which contain different nutrient concentration; as a result influence fungi growth and subsequent mycotoxins production. The bioavailability of nutrients in most grains would support the growth of a wide range of microorganisms. Although each strain of mold has the genetic potential to produce a particular mycotoxin, nutrient bioavailability could influence their levels significantly [45].

2.2 Biological structure

Grains have biological structures which prevent the penetration and growth of microorganisms. The testa of seeds and shell of nuts are examples of such structures. Some physical structures/barriers may exert antimicrobial potential. Intact biological structures prevent the entry of microbes, subsequent growth and production of mycotoxins in grains. However, these structures are destroyed during harvesting, transporting, or processing of the grains. Insect infestation could pave way for microbial proliferation of grains [46, 47]. Extract of Peanut testa was reported to exhibit pronounced antifungal activities against *Penicillium* sp., *A. niger*, and *Actinomucor* sp. The cardinal and purple peanut testa produced a significant zone of inhibition at concentrations of 0.8 and 2.0 g/L, respectively. It was concluded that the fungicidal potentials of the testa depend on the type of peanut [48]. Nevertheless, the environment, variety, type of farming system adopted, duration of storage, etc., may affect the fungicidal potency of these peanut testae.

The biocidal activities of *Dacryodes edulis* and *Garcinia kola* testae have been reported [49]. The antimicrobial activities of these testae are associated with the presence of phytochemicals (alkaloids, saponins, etc.), and was confirmed in experimental studies [50, 51]. The methanolic extract of *Simmondsia chinensis* testa (Link) C.K. Schneid exhibited no fungicidal activities against *Candida albicans* [52], indicating that not every grain testa could inhibit microbial growth.

All the studies mentioned above support the fact that the biological structures of the grains may have the potential to prevent microbial proliferation. These

claims cannot be guaranteed when the structures covering the seeds are destroyed during harvesting or drying. Therefore, care should be taken to minimize the destruction of these structures on grains during or after harvest. Busta et al. [53] reported that pathogens lack the enzyme necessary to break down the protective layers covering grains.

2.3 Moisture content (MC)

The oldest method of preserving food is controlling the MC. It is applicable during grain storage since the moisture influences the growth of microorganisms and subsequent production of mycotoxins. The water requirement of microbes is known as the water activity (a_w) of the food or environment and is defined as the ratio of the water vapor pressure of the food substrate to the vapor pressure of pure water at a constant temperature [47]. The a_w of grains describes the degree to which water is bound in the grains, its availability to participate in chemical/biochemical reactions, and its accessibility to facilitate the growth of microorganisms [53] which leads to the synthesis of metabolites.

Cereals have an a_w between 0.10 and 0.20 when adequately dried, making it difficult for microbes to reproduce. Although the optimum MC for growth and subsequent toxin production for the various aflatoxigenic fungi varies, many achieve the best growth and toxin synthesis at an MC of 17.5% [53, 54]. *Aspergillus* requires about 13% moisture or a relative humidity of 65% (a_w, of 0.65) for growth and toxin synthesis [55].

The highest *A. flavus* population was observed at a_w = 0.95. A_w significantly altered the AFB1 produced and the expression of *aflR* at a_w 0.90 and 0.95 respectively. The optimum expression of the *nor-1* gene was at a_w 0.95 and 0.90, whereas deficient expression occurred in the driest treatment (a_w 0.85) [56]. Molds were unable to germinate when the a_w of the grains remained below 0.60. Also, when molds are allow to flourish, they could predispose the stored grain to mite and insect infestation [3, 57] because mites feeds on molds. Co-culturing *A. parasiticus* with *S. lactis* and *Lactobacillus casei* suppressed aflatoxin synthesis [54]. In a similar study, Faraj et al. [58] reported a significant reduction in total aflatoxins synthesized when fungi (*A. niger* and *Rhizopus oryzae*) were co-cultured with a bacterium (*Bacillus stearothermophilus*). Since aflatoxins synthesis was minimal at 40°C and high between 8°C and 40°C, the authors associated the findings to the temperature differential between the strains [59]. However, mycotoxins such as rubratoxins from *Penicillium purpurogenum*, cerulenin from *Cephalosporium caerulens*, and *Acrocylindrium oryzae* inhibited fungi growth at the same time enhance aflatoxin synthesized [45, 60].

The growth of *Trichoderma asperellum* (strains PR10, PR11, PR12, and 659-7) was reported being sensitive to a_w reduction [61]. Therefore, lowering a_w could inhibit the growth of fungi. According to [62], grains stored for a year, 8–9 months, and weeks should have MC about 9%, 13%, and 14%, respectively. A low MC could curb problems like molds infestations, discoloration, respiration loss, insect damage, and moisture absorption.

Adequate drying of grains (produce) to lower moisture levels is critical to create unfavorable conditions to inhibit microbial and insect proliferation. It is recommended to dry harvested produce to safer moisture levels of 10–13%. Low moisture help keep grains longer without losing nutrients and other vital bioactive compounds [63, 64]. Water activity in stored grains could increase depending on climatic conditions, cellular respiration of microorganisms, or urine from rodents. Improper drying, especially during winter or autumn, could also elevate a_w levels.

2.4 pH, acidity and redox potential

For centuries, people have learned to increase the acidity of food either through fermentation, or by adding weak acids in the form of preservatives. These techniques have proven successful. Organic acids are effective preservatives in their undissociated state. pKa is the term used to illustrate the dissociation of an acid. Therefore, lowering the pH of grains increases the effectiveness of organic acids as preservatives [39, 53].

Naturally, grains in the field are undried and possess high pH; however, drying decreases the MC and subsequently the a_w, thereby reducing the pH. Adadi and Obeng [65] reported that the lower the pH value the higher the total acidity (TA), which inhibits the growth of microorganisms. The pH of grains could interact with other parameters (a_w, salt, temperature, redox potential) in the food to inhibit microbial growth. The general rule of food microbiology states that pathogens do not grow, or grow slowly, at pH below 4.6- but there are exceptions. For instance, at pH 4.2, an organism was able to survive and synthesize a mycotoxin [66].

Rice and maize have pH about 6.02 ± 0.01 and 6.53 ± 0.01 during the rainy season and 6.20 ± 0.20 and 6.42 ± 0.12, respectively, in the dry season [67]. The season seems to influence the a_w and the TA, thus altering the pH of the grains. The rainy season is defined by continuous rain, resulting in the elevation of the MC of the grains, which affects the pH. The pH range of beans (string and lima) is between 4.6 and 6.5 [53].

According to [68], fungi can secrete butyrate, oxalate, maleate, citrate, gluconate, and succinate into their environment, thereby changing the acidity of the ecological niche. *Sclerotinia sclerotiorum* and *Botrytis* sp. secrete oxalic acid while *Penicillium* spp., and *Aspergillus* spp., synthesize mainly gluconic and citric acids [69–71]. Fungi can grow comfortably in pH above 8.5; however, below pH 2.2, their growth was inhibited. Microorganisms can modify the pH of the environment in which they reside, making it challenging for farmers to control the pH of stored grain. A phenomenon like this could lead to significant economic loss due to microbial proliferation. The synthesis of ochratoxin A was maximized at lower pH [72]. Different fungi strains (*Trichoderma harzianum*, *Trichoderma aureoviride*, and *Trichoderma viride*) can grow over a broader pH range (from 2.0 to 6.0), with optimal growth at pH = 4.0 [73]. Hence, adjusting the pH is a great way of inhibiting the germination of any fungi spores on stored grains.

The redox potential (Eh) of a substance is the ratio of the total oxidizing (electron-accepting) power to the whole reducing (electron-donating) energy of the material. It is quantified in millivolts (mV) at pH 7.0. Eh correlates to the pH of a substrate [47]. Generally, aerobes, facultative anaerobes, and anaerobes grow well at Eh between +500 to +300 mV, +300 to −100 mV, and + 100 to less than −250 mV, respectively [74]. Some microorganisms require an Eh of less than +60 mV for growth; nevertheless, slower growth rates were observed at higher Eh values [53]. The Eh values of wheat (whole grain), wheat (germ), and barley (ground) is within −320 to −360, −470, and +225, respectively [46]. Oxidants such as $KMnO_4$, $NaClO_4$, or Fe_2O_3 can influence the Eh of a material [75]. The growth of *Fusarium oxysporum* and *Rhizoctonia solani* were suppressed when decomposable organic material was introduced [76, 77]. pH and Eh can impact a wide range of fungal physiological processes (regulation and expression of genes) [78–80] thus complicating the storage process. Therefore, controlling the Eh and pH of grains is necessary to manipulate fungi growth during storage.

2.5 Temperature

All microorganisms have a defined temperature range within which they can grow and synthesize toxins which cause food poisoning. Therefore, understanding the

temperatures range, coupled with other intrinsic and extrinsic factors, are crucial to selecte the proper storage conditions for grain storage. Temperature has a dramatic impact on the growth and lag period of an organism. The growth rates of most microorganisms are favored at low temperatures, though there are exceptions. Reaction rates for specific enzymes in an organism become slower at lower temperatures. Also, low temperatures minimize the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms in the cell [46, 53]. The expression of proteins are temperature regulated. A slight change in temperature can influence bacterial and archaeal community structure. 16S rRNA genes were altered due to changes in temperature [81, 82]. A wide range of temperatures play a vital role in the growth and synthesis of toxins in fungi. For instance, *Penicillium* and *Cladosporium* were able to grow below 20°C whereas the growth of *Aspergillus* species were inhibited. However, at a temperature above 20°C, the growth was maximized [55]. Virulent *A. niger* has optimal growth between 30–35°C [83], thus, rendering stored produce susceptible to a toxin secreted by these fungi. The growth rates of *Phoma* spp. 1, *Phoma exigua*, *Mortierella gamsii*, and *Mortierella* sp. 1 was high at 4°C [84]. Warmer (33°C) and more humid conditions may increase aflatoxin prevalence. However, the opposite scenario is expected in tropics, since most aflatoxigenic fungi will not survive the expected 40°C [45, 85].

The knowledge of optimal temperature for microbial growth and mycotoxin synthesis gives more accurate assessment of the potential risk to human health [72]. Molds can grow over a broader range of temperatures, from below freezing to temperatures over 50°C. For a given substrate, the rate of mold growth decrease with decreasing temperature and water availability. Below 17°C grains are susceptible to insect infestation; however, mite infestations can occur between 3 and 30°C [86]. Degradation of fungi mycotoxins can occur at 40°C [58]. Therefore, keeping the temperature of the storage room elevated could be of valuable aid in detoxification and probable killing of stored microorganisms.

3. Effects of mycotoxins on human health

Mycotoxins are considered a significant health and economic problem. Mycotoxins can find their way to the human body by way of contaminated food, skin contact, or inhalation [87, 88]. The most common form of exposure is through oral ingestion of contaminated food [89].

The level of exposure and the type of mycotoxins which one is exposed to determine the nature of adverse effects on the human, either in the form of an allergic reaction, infections, or a toxic disease [90]. The seriousness of mycotoxins depends on the toxicity of the mycotoxin involved, the age, wellbeing of the exposed individual, and the length of exposure [91]. Mycotoxicosis is the disease caused by mycotoxins. Mycotoxins such as aflatoxins have been documented causing liver cancer [92]. Other serious conditions, such as chronic interstitial nephropathy, Balkan endemic nephropathy, and urothelial tumors, as well as testicular cancer in men, have also been linked to mycotoxins [93]. Acute diseases, namely abdominal pains, headache, dizziness, throat irritation, and nausea, have also been associated with mycotoxin exposure in humans [94]. It is, therefore, important to ensure that grains are free of mycotoxin contamination.

3.1 Methods of detecting and analyzing mycotoxins

The hazardous effects of mycotoxins on humans and animals had called for the development of rapid methods for their detection and quantification in cereals

and other foods. However, sampling methods, extraction, and the instrument used could alter mycotoxin quantification. In response, Rahmani et al. [95] compiled a good comprehensive review to address the challenges mentioned above.

The impact of the sampling on sample preparation and analytical instrument contribute to the total variance during the analysis of ochratoxin A (OTA) in flour and aflatoxinB1 (AFB1) in oats was recently reported. The authors suggested that increasing sample weight (size) could potentially reduce the high heterogeneity encountered [96, 97]. For efficient extraction, methods of detection and quantification of mycotoxins, the reader(s) are referred to the following good sources [95, 98–101].

4. Some conventional methods of controlling grains microbial contamination

Contamination of stored grains by fungi mycotoxins has resulted in economic losses of food products, which could have been used to feed the less privileged (i.e., refugees, natural disaster victims, etc.). Therefore, preservation of grains during storage is necessary to maintain food security. Moreover, with the growing population of the world, more food will be required to feed folks. Some conventional approaches used in preserving grains are listed in **Table 1** besides those described below.

4.1 Organic acids (OA)

High-moisture grains are prone to deterioration during storage if moisture exceeds 14%. For this reason, in the 1970s, chemicals were used to preserve high moisture grains. Propionic acid was used alone (applied worldwide) or in combination with acetic acid, isobutyric acid. Formaldehyde was mostly used in Europe to inhibit the growth of mold and bacteria in outdoor storage of grains. However, when galvanized steel equipment are used to store acid treated grains, extreme corrosion occurred. Thus, lining the bins with oil was recommended. The combinations of propionic acid and sodium benzoate curbed the issue of corrosion, and less harmful compared to pure propionic acid [114–116]. Coating the bins with silver nanoparticle protective paints [117] could prevent corrosion and exert fungicidal activities.

Reference	Methods	Limitations
[4, 102, 103]	Debranning	• Not entirely suitable for wheat due to the crease on the wheat kernels. • Whole-grain demand in the market.
[104–106]	Pesticides	• High environmental impacts. • Direct negative impact on human health. • Increasing resistance against pesticides.
[107–110]	Ozone	• The cost of treatment can be relatively high due to complex technology. • Limited to highly vented packages or open-top containers.
[111–113]	Irradiation	• Can negatively modify the quality and technological properties of cereals and cereal products

Modified with permission from ref. 4496530764014 [122].

Table 1.
Some conventional approaches of grains preservation.

OA can increase moisture content and penetrate the endosperm, thus alter the functionality of the grains [118, 119]. It could also modify the nutritional composition of the stored grain, consequently decreasing the quantity and quality of nutrients. The combination of organic acids, such as propionic, sorbic, and acetic acids, as well as their salts, had antimould activities, which extended the shelf life of bakery products [36]. Similarly, calcium propionate (0.003%), potassium sorbate (0.03%), and sodium benzoate (0.3%) suppressed the growth and mycotoxin production in *Eurotium, Aspergillus* and *Penicillium*. However, the author claimed that a_w and pH contributed to the effectiveness of the compounds and should therefore be carefully considered during application [115]. High sorbate concentration altered the sensorial properties of food [120]; therefore, the concentration used is crucial to maintain grain quality after storage. Propionic acid and its salts exhibited antimicrobial effect against *Bacillus* spp., and was ascribed to their high MW fatty acids [120]. Valerio et al. [121] tested the antifungal activities of organic acids synthesized by lactic acid bacteria (LAB) isolated from a semolina ecosystem. The results showed that all the acids produce by the LAB had inhibitory effects on the test species (*Penicillium roqueforti*, *A. niger*, and *Endomyces fibuligera*). This approach could be classified as biopreservation since the metabolites of living organisms were used to inhibit the growth of microorganisms on the product.

4.2 Drying

According to [122], drying is the phase of postharvest processing during which grains are dried to achieve low MC, thereby guaranteeing safe storage (<0.70 a_w). The MC of adequately dried grains ranged within 10–14%. Russ and coworkers [123] reported that at higher MC, residue of fermentable sugars and other nutrients predispose grains to microbial colonization, resulting in rapid deterioration. Thus, a productive drying process warrants the reduction of moisture, thereby lowering the pH and creating an uninhabitable environment for the germination and proliferation of a microorganism. Dried grains should be allowed to cool before bagging because heat generated during drying could cause a warm spot. Earlier works [36] reported that warm spot in grains support fungal growth, resulting in contamination of grain by mycotoxins. Kumar and coworkers [124] reviewed a paper on heat convection solar drying systems. Some of the techniques described could be employed when drying grains. The low-cost material utilized in manufacturing these dryers, coupled with user friendly, make them ideal for large scale drying, even for small-scale farmers.

Different drying methods have been described: (1) high temperature or heated air-drying; (2) low-temperature air-drying; (3) combined air-drying; (4) dry ration and in-storage cooling method (an alternative to in-dryer cooling) [125, 126].

The expensive nature (cost of power) of artificial drying makes it unpopular, couple with the technicalities involved. For instance, in Russia, sun drying becomes insufficient due to the high MC (i.e., in St Petersburg, Yekaterinburg, etc.); thus, it is impossible to achieve uniform drying of grains. In Africa, sun drying is efficient and effective since there is almost 13-h of sun during the dry season [127]. Applying excessive temperatures (using artificial means) can lead to grains cracking, loss of viability, as well as economic losses [122, 128].

4.3 Chlorine and hypochlorite

Chlorine dioxide (ClO_2) has biocidal activities due to its oxidizing capacity (strong oxidant), and is widely used for decontamination. It is used both in its gaseous and aqueous forms to sanitize food and, exert potent biocidal activity against

bacteria, yeasts, and molds [129–133]. All bacteria and their spores in a hospital room were reported killed/inactivate by ClO_2 gas [134].

Poliovirus was found to have been inhibited due to the application of ClO_2, which interreacted with the viral RNA and damaged the genome's ability to act as a template for RNA synthesis [135]. Aqueous ClO_2 was documented to have significantly enhanced the inactivation of *F. graminearum* on wheat at high concentration, (15 mg/L) compared to lower levels (5 and 10 mg/L) [131]. Inexpensive, less corrosive, the ease with which it mixes with air, rapid diffusion, and being easy to use are some merits associated with this method. However, it can produce toxic by-products and interfere with the flavor compounds in the grains. It also requires expensive onsite generation [136–139]. Chlorine solution (0.4%) was ineffective against highly contaminated grains [140, 141]. The reason could be the colonies were mature and had thicker peptidoglycan, hence, the chlorine could not penetrate the cells to reach the genetic material. Another hypothesis could be that the concentration was not enough to destabilize cell and react with the amino acids. Sun and collaborators [133] documented that coupling aqueous sanitizer with gaseous ClO_2 enhanced the decontamination of foodborne and plant pathogens. It also improved the safety, quality, and sensory properties of products (fruits and vegetables). Nevertheless, higher concentrations may cause bleaching or browning.

5. Nanoparticles

The term 'nano' is a Greek word for dwarf, and a nanometer (nm) is 1-billionth of a meter. Nanotechnology has been in existence for decades now, and not an invention of the twentieth century. Nanomaterials and nanoparticles (NPs) are materials that have at least one dimension on the nanoscale (1–100 nm) or whose basic unit in the three-dimensional space is in this range. NPs have a more comprehensive range of applications in food science and technology, drug delivery, biomedical engineering, tissue engineering, textile industry, environment, electronics, agriculture, etc. [10, 142–145]. Nanoparticles are classified as organic (also known as nanocapsules) and inorganic.

Organic NPs act as core shells to shield sensitive bioactive ingredient such as carotenoids [146] against environmental factors, thereby enhancing their bioavailability for safer delivery [10, 147]. Nanoprecipitation, emulsion-diffusion, double emulsification, emulsion-coacervation, polymer coating, etc. are examples of organic NPs [148]. All these techniques are used to prepare the core materials (β-carotene, probiotic bacteria, folic acid, omega fatty acid, protease enzymes, etc.) for encapsulation. Fluorescent organic NPs have recently been used to develop nanosensors [149] which are used to detect contaminants and other foodborne pathogens as well as in bioremediation [150].

Inorganic NPs have attracted the attention of researchers in the last two decades due to their multiple antimicrobial activities (antifungal or antiviral) coupled with the pronouncement from Food Safety Authority that these NPs are safe and do not affect humans/consumers in any way [151–153]. Silver, silica, and titanium dioxide NPs are the main NPs used in the agri-food industries [154].

5.1 Silver nanoparticles (AgNPs)

Several studies have confirmed the potent biocidal effects of silver nanoparticles (AgNPs) towards fungi [155–158]. Due to their peculiar properties (i.e., optical,

electrical, and thermal, and biological properties), AgNPs have been used in several applications: as biocidal agents; medical device coatings; optical sensors; in cosmetics; in the food industry (food products); in diagnostics, orthopedics, drug delivery; as anticancer agents and have greatly enhanced the tumor-killing effects of anticancer drugs [158–163]. Healthcare products, such as scaffolding, burn dressings, water purification systems, and medical devices are manufactured using AgNPs [164, 165]. It was reported that 10 μg/mL AgNPs completely inhibited the growth of 10^7 CFU/mL *E. coli* ATCC 8739 cells in liquid medium. The leakage of reducing sugars and proteins forced respiratory chain dehydrogenases into an inactive state, suggesting that AgNPs penetrated the bacterial cell membrane with high efficiency and could therefore be used in the manufacturing of drugs used against bacterial diseases [158]. AgNPs extracted from *Pistacia atlantica* were effective against important clinical pathogens [166]. AgNPs synthesized (green AgNPs) from the leaf of CRCP (medicinal plant) was utilized against multidrug-resistant (MDR) *P. aeruginosa*, *S. aureus* and CoNS isolates (10^6 CFU each) from post-surgical wound infections. 80 mg/mL AgNPs was reported effective against, *S. aureus* and CoNS isolates but had little effects on *P. aeruginosa*. However, 100-120 mg/mL AgNPs completely inhibited *P. aeruginosa* [153]. These findings shows that the concentration of AgNPs utilize is critical therefore should carefully be considered during application.

The fungicidal activities of AgNPs are documented in many studies [13, 152, 160, 167–170]. Six fungal species (*Aspergillus fumigatus*, *Penicillium brevicompactum*, *Cladosporium cladosporoides*, *Mortierella alpina*, *Chaetomium globosum*, and *Stachybotrys chartarum*) isolated from an indoor environment were used to test the antifungal activity of AgNPs. The results revealed that the presence of AgNPs in concentrations of 30–200 mg/L significantly inhibited or decreased the growth of all the fungi species except *Mortierella* species, which were insensitive to the AgNPs but instead metabolized the AgNPs for its own benefit (the presence of AgNPs in agar substrates significantly enhanced *Mortierella* growth rate) [152]. AgNPs and a conventional antifungal agent, Amphotericin B (for a positive test), were tested against *Saccharomyces cerevisiae* (KCTC 7296), *Trichosporon beigelii* (KCTC 7707), and *Candida albicans* (ATCC 90028). The AgNPs exhibited a minimum inhibition concentration (MIC) value of 2 μg/mL, similar to the positive control [155]. AgNPs was found to effectively suppress growth and AFB1 production in *A. parasiticus* (**Figure 1**) [171]. In a similar study, the addition of AgNP HA1N, AgNP HA2N, and AgNP EH resulted in 88.2%, 67.7% and 83.5% reduction of AFB1 synthesized by *A. flavus* [172]. Also, the fungicidal activity of *Capsicum annuum* L. was recently reported [173]. The active ingredient could be isolated and encapsulated in NPs, which may exhibit potent inhibitory activities against storage pest and microorganism.

5.1.1 Mechanistic action of AgNPs biocidal activities

The potent antimicrobial activity of AgNPs has attracted global attention, hence its application in multiple fields (i.e., food industries, medicine, textile industries, etc.). However, the exact mechanistic action is still not clear, because the mechanism depends on the type of microorganism (i.e., bacteria, fungi, etc.) involved and, since different organisms possess different cell structure, the mechanistic action differ. Several researchers have tried to understand the antimicrobial effects of AgNPs using various model microorganisms, e.g., *E. coli* [158, 174, 175], *P. aeruginosa, S. aureus* [175], *V. cholera* [174, 176], *S. cerevisiae* [177, 178] and *S. typhi* [174]. Other groups [179, 180] have also worked on fungi. Mitochondrial dysfunction predispose cells for easier penetration by AgNPs via diffusion and endocytosis. The efficiency of

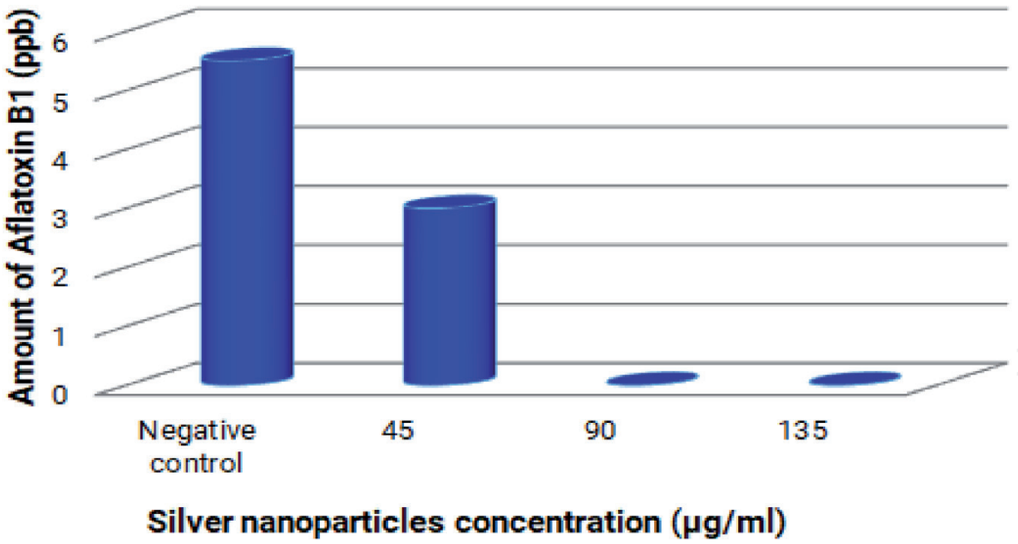

Figure 1.
Inhibition of aflatoxin B_1 production at different concentration of AgNPs. Modified with permission from © Iranian Journal of Medical Sciences [171].

AgNPs uptake by skin keratinocytes depends on the size, shape, pH, zeta potential, and incubation time. Smaller (<5 nm) NPs are more toxic than the larger ones. This could be ascribed to the secure attachment and penetration of the smaller NPs compared to the larger NPs, which requires larger pores to penetrate, into the cell membrane and internalized. AgNPs were able to attach and penetrate cell membrane causing toxicity in *Caenorhabditis elegans*. Ag^0 can interact with molecular oxygen, as well as with other redox-active compounds to produce ionic silver, which then further interact with environmental factors to yield Ag^+ [181–186]. AgNPs ranging from less than 10 nm can inhibit *E. coli* and *P. aeruginosa* due to their potent biocidal activities [187, 188]. Certain viruses were unable to bind to their host cells due to the presence of AgNPs of 1–10 nm, thus starving them to death [189]. Concerning shapes, Pal et al. [190] reported that triangular AgNPs were found to be effective compared to rod and sphere AgNPs. The biocidal efficiency of AgNPs is related to Ag^+, which interact with biological macromolecules (proteins, carbohydrates, nucleic acids, and lipids). When AgNPs adhere to the surface of the cell, it automatically alters membrane properties, undermining the fluidity of the cell. AgNPs can degrade lipopolysaccharide molecules causing them to accumulate inside membrane by forming "pits", thereby increasing membrane permeability [191]. According to reports Ag^+ can inhibit phosphate uptake, resulting in the efflux of phosphate, mannitol, succinate, glutamine, and proline from the cell [192–198].

The minimal bactericidal concentration (MBC) of AgNPs on Gram (+) bacteria was 32 times higher compared to Gram (−) cells [199]. Thus, the sensitivity of the cell wall depends on the class of microorganisms. Research [174] also demonstrated that AgNPs can interact with bacterial cell membranes. Furthermore, the AgNPs found inside the cells are the same sizes as the ones interacting with the membrane, therefore providing more evidence to support the theory that particles that interact with the membrane penetrated into the bacteria.

Several studies [176, 200, 201] have reported that the positive charge of AgNPs is crucial for its antimicrobial activity through the electrostatic attraction with the negatively charged cell membrane of the microorganism.

The permeability of the cell membrane was altered after treatment with AgNPs, resulting in the leaking of reducing sugars and proteins which induced respiratory chain dehydrogenases into inactive state. The amount of reducing sugars leaked after 2 h was 102.5 and 30 µg/mg per bacterial dry weight in the treated and the control cells, respectively. While the activity of respiratory chain dehydrogenases

of positive control increased at 37 ± 2, nearly no change was observed in negative control cells. Furthermore, the enzymatic activity of cells treated with 5 μg/mL AgNPs decreased [158]. The survival rate of bacterial species decreased with increase in the adsorption of AgNPs. Additionally, the adsorption and toxicity of AgNPs on *P. aeruginosa*, *M. luteus*, *B. subtilis*, *B. barbaricus*, and *K. pneumonia* was optimum at pH 5, NaCl concentration of <0.5 M. A manifestation of less toxicity was noticed at pH 9 and NaCl concentration >0.5 M, indicating that the environmental pH under which the microorganism grows plays a crucial role in either protecting or exposing it to rapid interaction with the AgNPs [185]. The ability of AgNPs to bind, interact, deform, and induce DNA damage was documented [181, 202–204]. Hackenberg and coworkers [203] used comet assay and chromosomal aberration (CA), a method previously recommended by [205], to determine the damage AgNPs inflict on DNA. In both methods, maximum damage to human mesenchymal stem cells occurred less than an hour after treatment (0.1 μg/mL). Circular dichroism spectra analysis of treated calf thymus DNA revealed that AgNPs interacted and formed a new complex with the double-helical DNA, then induced an alteration of non-planar and change the orientations of DNA bases which act as an intercalator, increasing the stability of DNA which in turn increase the Tm value of the DNA [202]. A researcher [206] suggested that AgNPs can interact with nucleic acids by forming bonds with pyrimidine bases, thus condensing DNA and inhibiting replication. In a recent study, Li et al. [207] showed that citrate-AgNPs (C-AgNP20) induced different cytomorphological alterations and intracellular distributions in cetacean (bottlenose dolphins (*Tursiops truncatus*)) polymorphonuclear cells (cPMNs) and peripheral blood mononuclear cells (cPBMCs). High dose (10 and 50 μg/mL) of C-AgNP20 triggered apoptosis in cPMNs and cPBMCs (induced cytotoxicity). Additionally, the functional activities of cPMNs (phagocytosis and respiratory burst) and cPBMCs (proliferative activity) were negatively altered at sub-lethal dose of 0.1 and 1 μg/mL. AgNPs induced structural damage to cell wall, intracellular proteins (enzymes), and organelles, leading to the disruption or the collapse of metabolic processes, like antioxidant defense mechanisms, thereby inhibiting growth [177, 178].

The cellular oxidative stress in microbes was enhanced by increasing the concentration of Ag (+) ions [206]. Several reports [208–213] have highlighted the potential antiviral, antifungal, and antibacterial activities of AgNPs and was ascribed to its ability to generate enough reactive oxygen species (ROS), free radicals (i.e., hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hydroxyl radical ($OH^{\bullet}$), hypochlorous acid (HOCl)) and singlet oxygen. During mitochondrial oxidative phosphorylation, ROS are produced. Moreover, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyzes series of reactions where molecular oxygen (O_2) is reduced to $O^{2\bullet}$. With dismutation and metal-catalyzed Fenton reaction, the $O^{2\bullet}$ is further reduced to H_2O_2 and $OH^{\bullet}$, respectively [214–216]. Apoptosis and cell membrane damage were induced by ROS, leaving the cells incapable of regulating transport through the plasma membrane, resulting in cell death [217–220]. A research group [221], evaluated the effects of ROS against *S. aureus* and *E. coli*. The results showed the inactivation of lactate dehydrogenase and protein denaturation in both test organisms. Membranal damage allowed influx of calcium, thus inducing intracellular calcium overload, further doubling ROS generation and mitochondrial membrane potential variation [222]. The overproduction of ROS was reported to have interfered with ATP synthesis, leading to DNA damage [223]. Free radicals and ROS (an excessive amount) can inflict damage/stress on the mitochondrial membrane, causing necrosis, peroxidation of lipids, proteins, and DNA damage [206, 224, 225]. According to [184, 225], elevated levels of ROS can stress the endoplasmic reticula and deactivate antioxidant enzymes in cells, resulting in genotoxic effects.

It has been discovered that $OH^{\bullet}$, interacted with constituents of DNA, which led to the breakage of DNA single-strands via the formation of 8-hydroxyl-2′-deoxyguanosine (8-OHdG) DNA adduct [226, 227]. In vivo studies have shown that AgNPs influenced the activity of chicken oxidative stress enzymes [228]. AgNP treatment induced a pronounced ROS in *P. aeruginosa* compared to $AgNO_3$. The expression levels of ROS related proteins (PA4133, Hmp, KatA, CcoP2, SodB, CcpA, RibC, EtfA, and PiuC) were specifically regulated after exposure to AgNPs in concentration and time-related modes. Cells treated with $AgNO_3$ did not show any perturbation in intracellular ROS generation at low levels, which supports the existing theory that oxidative stress is triggered solely by AgNPs at their corresponding concentrations [229]. As reported by [220], the biocidal activities of Ag^+ could also be attributed to its interactions with the thiol-related compounds found in the respiratory enzymes of cells, resulting in cell death. A researcher [230] proposed a theory using Ag with cellular energy production. Essential proteins of prokaryotes and eukaryotes located on the cell exterior and interior (mitochondrial organelles), respectively, deactivated after coming in contact with AgNPs. However, the interior components (mitochondrial proteins) required higher concentrations and much smaller AgNPs before they are rendered inactive, because the cellular membrane acted as a diffusion barrier. Moreover, the eukaryotes possessed numerous biological energy conservation system due it extensive mitochondria when compared to the prokaryotes, thereby predisposing the latter cells to AgNP interaction, hampering cell respiration, which led to cell death.

6. Conclusions

It is shown from the above studies that all the mentioned microorganisms, especially the fungi, are involved in grain contamination and subsequent mycotoxin production during storage. Mechanical damage during harvesting or processing served as an easy route via which microorganisms penetrated the endosperms of seeds, and secrete mycotoxins (aflatoxins, etc.) rendering stored grains unsafe for human consumption. The ability of AgNPs to inhibit microbial growth makes them a promising candidate for utilization in storing grains to minimize the economic losses and food poisoning caused by mycotoxins contamination. Moreover, AgNPs inhibited the synthesis of these mycotoxins by switching off molecular pathways via which they are produced, thus guaranteeing the safety of stored grains for consumption. The utilization of AgNPs could enhance shelf-life, maintain the quality and nutritional values of grains. This innovative method is safe and do not pose a threat to the consumer or the environment.

Acknowledgments

This work was supported by the Ministry of Science and Higher Education of Russian Federation, goszadanie no. 2019-1075.

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